Review

Heat stress preconditioning and delayed myocardial protection: what is new?

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Abstract

As other preconditioning phenomena, heat stress is able to induce a delayed myocardial protection against ischaemia-reperfusion injury by preserving ventricular function, preventing arrhythmia occurrence and reducing cellular necrosis. The development of heat stress response has been extensively studied in order to characterize the different steps of this form of preconditioning. It appears that chemical signals (such as nitric oxide, reactive oxygen species (ROS)) released by sublethal hyperthermic stress trigger a complex cascade of signalling events that include activation of protein kinase C (PKC) and mitogen-activated protein kinases (MAPK) and culminate in increased synthesis of inducible nitric oxide synthase, cyclooxygenase-2, antioxidant enzymes and protective proteins such as heat stress proteins (Hsps). A better understanding of this powerful protective adaptation of the cardiomyocyte is essential for the development of clinical applications and the design of cardioprotective pharmacological agents. The purpose of this letter is to review current information regarding the characteristics of heat stress preconditioning compared to other forms of late preconditioning.

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1. Introduction

Although prevention of atherosclerosis has resulted in a significant decrease in the incidence of acute myocardial infarction in the past decade, it is still the most common cause of death in man in the Western World. Thus, understanding the nature of myocardial ischaemia and elucidating endogenous cardioprotective mechanisms in order to develop rational modes of therapy remain of primary importance.

The heart possesses a remarkable ability to adapt to stress by changing its phenotype in a manner that renders it more resistant to subsequent injury. This powerful adaptive phenomenon, called preconditioning, is illustrated by the fact that a sublethal stress (such as ischaemia or heat stress (HS)) applied to the myocardium enhances its tolerance to a subsequent ischaemic stress [1,2].

The delayed transient cardioprotection, occurring 24–48 h after HS, results in a significant myocardial salvage following coronary occlusion and reperfusion [3,4]. If the mechanism by which myocardial cells adapt to stress was understood, design and testing of specific pharmacological agents that activate this mechanism can hopefully follow.

In this review article, we therefore discuss in detail the endogenous protective mechanisms occurring within the heart during HS preconditioning, from its initiation by different chemical signals triggering a complex cascade of signalling events, to the mediation of cardioprotection by many potential candidates. We also discuss how recent data suggest that adaptation to stress represents a new direction for myocardial protection using to our best advantage the ability of the cell to self-protect.

2. Induction of myocardial HS preconditioning

HS represents a non-pharmacological preconditioning, such as ischaemic preconditioning or exercise. It was the
first stress shown to induce the synthesis of heat stress proteins (Hsps), in particular the inducible form Hsp70, in the heart and other tissues [5]. It usually consists of a whole body hyperthermia during which the rectal temperature of the experimental animal is maintained at 42 °C for 15 min [3]. More recently, local heating of the heart in vivo has been proposed to prevent extracardiac sequels occurring during whole body hyperthermia [6]. In cultured cardiomyocytes, HS is performed using an increase of ambient temperature, inducing cellular Hsp70 expression and cytoprotection [7].

3. End-points of HS preconditioning

HS preconditioning can protect the myocardium against various types of stresses [3,8], but because of its clinical relevance, we only review in this section the aspects of protection against ischaemia-reperfusion injury.

3.1. Preservation of left ventricular function

Currie et al. [3] were the first to show that 24 h after HS, isolated rat hearts exhibited improved contractile recovery upon reperfusion compared to control hearts. Since then, this observation has been extended to the rabbit [9] and the dog [10]. This effect seems to be age-dependent, since HS-induced improved postsischaemic functional recovery is observed only in young adult rats and disappears in aged ones [11,12].

3.2. Antiarrhythmic effect

In the rat, prior hyperthermia reduces the incidence and duration of ventricular arrhythmias following a short sequence of ischaemia-reperfusion, both in vivo and in vitro [13,14]. This confirms that the antiarrhythmic effect of HS does not involve a circulatory agent and can be explained by protective mechanisms originating within the myocardium.

3.3. Infarct size reduction and enhanced cellular viability

The effect of HS on these end-points is of particular clinical relevance. Donnelly et al. [4] were the first to show, in vivo in the rat, that HS reduces infarct size induced 24 h later by a 35 min left coronary artery occlusion—120 min reperfusion sequence. Since then, this result has been confirmed in the rabbit [15,16] and the mouse [17]. Moreover, we have shown that HS is able to protect hypertrophied myocardium from transgenic (tmREN-27) hypertensive rats against infarction [18]. In accordance with these observations, HS is also able to enhance the viability of isolated cardiomyocytes submitted to metabolic conditions mimicking ischaemia in vitro [7,19].

Moreover, various markers of cell injury, such as creatine kinase [3] and lactate dehydrogenase release [17], are also reduced by HS preconditioning. HS is also able to preserve mitochondrial energetic capacity and structure against ischaemic injury, an effect potentially dependent on the synthesis of mitochondrial Hsps [20]. Finally, HS has been shown to improve the metabolic status of reperfused myocardium by preserving high-energy phosphate levels [9].

3.4. Preservation of coronary endothelial function

We [21] and others [22] have observed that HS preconditioning can prevent the endothelial coronary dysfunction induced by ischaemia reperfusion [23].

4. Components of the mechanism of HS preconditioning

The delayed protection against ischaemia induced by HS is the result of a complex cascade of cellular events representing an archetypical response of the heart to stressful stimuli. Conceptually, it is useful to subdivide this response into three major components: (i) the chemical species that are generated during HS and initiate the preconditioning (triggers), (ii) the signalling pathways that are activated by the triggers and lead to the cardioprotection and (iii) the molecular species that are expressed and confer protection 24–48 h later (mediators). These potential actors of HS response have been identified using pharmacological tools applied either during HS (assumed to interfere with triggers) or at the time of ischaemia (assumed to interfere with mediators). This approach has some limitations, in particular, the specificity of inhibitors is often a matter of serious concern.

4.1. Triggers of HS preconditioning

Hyperthermia results in the generation of a wide variety of metabolites and ligands, reviewed in this section, which trigger the development of cardioprotection by switching the phenotype of cardiomyocytes to a defensive one (Fig. 1).

4.1.1. Catecholamines

In the conscious rat, plasma catecholamine concentrations and myocardial noradrenaline turnover have been shown to increase during HS [24,25]. Moreover, we have demonstrated that α1-adrenoceptors play a role in the HS response since antagonism by prazosin during HS abolishes delayed resistance to myocardial infarction [26]. Taken together, these results suggest that catecholamines could be involved in triggering HS preconditioning.

4.1.2. Nitric oxide

The most abundant free radical in the body, nitric oxide (NO) [27], is also able to initiate HS preconditioning. Indeed, HS sharply increases NO production in different
rat organs including the heart [28]. Furthermore, we have recently shown that the delayed reduction in infarct size observed in isolated rat hearts is abolished by administration of the iNOS inhibitor L-NIL prior to HS [29].

4.1.3. Reactive oxygen species

Hyperthermia also induces the production of reactive oxygen species (ROS), such as superoxide anion (O$_2^-$) [30,31], which also participate in the initiation of HS response. We have recently shown that oxidative stress occurring upon HS can trigger preconditioning since MPG pretreatment prevents the HS-induced infarct size reduction in the isolated rat heart [32].

Further studies will be necessary to determine the source and identity of the ROS responsible for initiating HS preconditioning and to assess whether NO and ROS are part of the same mechanism (i.e., whether the involved reactive radical species are derived from the reaction of NO with O$_2^-$) or act in parallel as two independent triggers [33]. Indeed, NO is known to react rapidly with O$_2^-$ to form the peroxynitrite anion (ONOO$^-$), which decomposes, generating various highly reactive oxidants such as the hydroxyl radical (•OH) [34]. Moreover, peroxynitrite is able to induce nitration of structural proteins creating nitrotyrosines, an effect with potential consequences on intracellular signalling [35,36].

4.1.4. Cytokines

Plasma levels of different cytokines such as interleukin-1β (IL-1β), interleukin-6, interferon-γ and tumor necrosis factor (TNF) are also increased following hyperthermia [37]. Moreover, HS-induced cardioprotection is abolished by administration of neutralising antibodies to IL-1β and TNF-α before HS [38]. ROS generation during hyperthermia has been shown to increase myocardial IL-1β and TNF-α levels [39], leading to rapid activation and nuclear translocation of the transcription factor nuclear factor-κB (NF-κB) [38,40]. However, the participation of NF-κB in the HS response remains to be investigated.

4.1.5. Heme oxygenase-1 pathway

Heme oxygenase-1 (HO-1, also regarded as Hsp32), whose expression is markedly increased 4–16 h after HS [41,42], is an HO isoform which degrades intracellular heme into carbon monoxide, an important signal messenger regulating cardiovascular function, and bilirubin, a potent antioxidant. Since HS-induced cardioprotection can be abolished by treatment with an HO inhibitor prior to hyperthermia, a role for the HO-1 pathway in triggering HS preconditioning can be evoked [42].

HS is also able to activate capsaicin-sensitive sensory nerves and stimulate the release of neurotransmitters including calcitonin gene-related peptide (CGRP), which is in-
involved in HS-induced cardioprotection [43,44]. Since delayed cardioprotection afforded by pharmacological preconditioning has been shown to be triggered by CGRP via activation of the HO-1 pathway [45], this might also be the case for HS-induced cardioprotection, although this hypothesis remains to be verified.

4.1.6. Opioids

Recent data in rats indicate that activation of δ1-opioid receptors is also involved in triggering the HS response, since delayed cardioprotection is abolished by the opiate receptor antagonist naloxone when administered before hyperthermia [46]. Further studies using knock-out animals for δ1-opioid receptors are needed to confirm this hypothesis.

4.2. Signalling aspects of HS preconditioning

The stimuli previously cited trigger HS preconditioning by activating a complex cascade of signalling events that ultimately result in increased transcription of cardioprotective genes. Over the last years, the exploration of these signalling pathways has been undertaken and some key steps have been identified (Fig. 1).

4.2.1. Phospholipase C and protein kinase C

Intracellular 1,4,5-inositol triphosphate is released by HS [47], an effect antagonised by phospholipase C inhibition. Activation of phospholipase C also leads to diacylglycerol release and protein kinase C (PKC) activation. We have indeed demonstrated that PKC inhibition by chelerythrine prior to HS abolishes the cardioprotection induced 24 h later in the isolated rat heart [48], a finding in accordance with an in vivo study [49]. Moreover, recent observations suggest an important role for the epsilon isoform of PKC in this cardioprotective mechanism [50], which can be activated by NO [51,52]. Since we have shown that iNOS-produced NO can trigger HS preconditioning [29], we can postulate that NO production could be responsible for PKC activation upon HS, a hypothesis that remains to be confirmed.

4.2.2. Protein tyrosine kinases

We have reported that tyrosine kinases did not appear to be involved in HS preconditioning since inhibition by genistein prior to hyperthermia did not affect cardioprotection induced 24 h later in the isolated rat heart [48]. However, this remains to be confirmed since HS has been shown to activate c-Src tyrosine kinases in fibroblasts [53].

4.2.3. Mitogen-activated protein kinases

In response to different stresses including HS, p38 mitogen-activated protein kinase (MAPK) is activated through dual phosphorylation on Thr-180 and Tyr-182 residues [54]. As shown by in vitro assays [55], two p38 MAPK substrates (MAPKAPK-2 and -3), phosphorylate Hsp27, a potential mediator of HS preconditioning.

In exploring the signalling pathways of HS preconditioning, we have shown that HS-induced myocardial ischaemic tolerance in the rat was abolished by the p38 MAPK inhibitor SB 203580 administered prior to hyperthermia [56]. This was also observed in the mouse [17]. However, we and others were unable to demonstrate a p38 MAPK phosphorylation after HS [17,50,56]. Further investigations, with knock-out animals or more specific pharmacological agents, are required to elucidate the precise role of various MAPK families in HS preconditioning and the separate or potentially coupled transduction pathways in which they are involved. In particular, MAPKs could represent potential downstream targets of PKC-dependent signalling mechanisms [57,58].

4.3. Mediators of HS preconditioning

HS preconditioning requires increased synthesis of new proteins to induce cardioprotection. Indeed, the time course of enhanced tolerance to ischaemia, which requires 24–48 h to develop and lasts for 3–4 days [59], is also consistent with the synthesis and subsequent degradation of cardioprotective proteins. Several proteins, which are reviewed in this section, have been proposed as potential mediators of the protection afforded by HS preconditioning (Fig. 1). Most of the mediators cited seem to play a role in the final steps of the signalling pathway associated with the HS response. But at least two of them, the antioxidant enzymes and changes in calcium homeostasis, could represent potential final end-effectors in the mediation of cardioprotection induced by HS preconditioning.

4.3.1. Heat stress proteins

HS induces an increase in expression of various Hsps (Hsp110, Hsp90, Hsp70 and small molecular mass Hsps) that could all be responsible for protection against myocardial ischaemia [60]. In particular, members of the Hsp70 family have been shown to repair or remove denatured proteins within the cell, leading to restoration of cell function during recovery from stress [61]. The evidence suggesting Hsp70 as the primary mediator of cardioprotection was brought about by the observation of a direct correlation between the amount of Hsp70 induced following HS and the degree of myocardial protection in the rat [62] and in the rabbit [63]. Further evidence that Hsp70 plays a direct role in the protection from myocardial ischaemia-reperfusion injury has been obtained using transfected cultured cells [7] [64] or animals [65–67] overexpressing the Hsp70 gene.

Although these studies demonstrate the cytoprotective effects of Hsps, the direct relationship between Hsp synthesis and HS-induced cardioprotection remains controversial. Indeed, several recent studies indicate that the quantitative accumulation of Hsp70 is unlikely to be the sole determinant of HS-induced cardioprotection since it occurs independently of the level of Hsp70 expression [17,49,68]. Also,
we and others have shown that the infarct size-reducing effect of HS is abolished by α1-adrenoceptors blockers [26] and by PKC [48,69] and MAPK inhibitors [17,56], without concomitant changes in Hsp70 induction. These observations reinforce the hypothesis that several cytoprotective mechanisms are involved in HS preconditioning. Finally, the main protective role of chaperoning HspS could thus be to bind and protect other potential end-effectors.

4.3.2. Nitric oxide

We have provided the first demonstration that iNOS-derived NO is a mediator of HS preconditioning, since the infarct-sparing effect seen in vivo in the rat is abolished by two NOS inhibitors, L-NAME and 1400W, when given before ischaemia [70]. Concurrently, we have shown a strong increase in iNOS protein expression 24 h after HS. Activation of the iNOS gene transcription by preconditioning has been linked to various upstream components (see Section 4.1.) such as NO itself [71] or NF-κB [72].

4.3.3. Cyclooxygenase-2

Cyclooxygenase-2 (COX-2) catalyses the first two steps in the biosynthesis of prostaglandins (PGs) from arachidonic acid [73]. We were the first to observe that COX-2 activity is necessary during ischaemia-reperfusion to mediate cardioprotection, since the protective effect of HS is abolished by two different COX-2 inhibitors (celecoxib and NS-398) when given before ischaemia [74]. We have also shown a marked increase in myocardial COX-2 protein expression 24 h after HS.

COX-2 can be activated by NO [75], but this remains to be investigated in the context of the HS response.

4.3.4. Endogenous cannabinoids

The first evidence of the implication of endogenous cannabinoids in mediating HS-induced cardioprotection has recently been provided by our group. Thus, in isolated rat hearts, perfusion by a CB2 receptor antagonist (SR 144528), but not by a CB1 receptor antagonist (SR 141716), abolishes the infarct size-reducing effect of HS [76].

The endocannabinoid system, which is related to NO production [77], appears to be involved in the regulation of many cardiovascular functions, with endocannabinoids being able to induce hypotensive and bradycardic effects (for a review see Ref. [78]).

4.3.5. K\textsubscript{ATP} channels

The opening of ATP-sensitive potassium (K\textsubscript{ATP}) channels appears to play a role in mediating HS preconditioning in the rat [79] and in the rabbit [80,81]. In particular, it has been shown that the mitochondrial K\textsubscript{ATP} channel blocker 5-hydroxydecanoate is able to abolish the HS-induced cardioprotection.

PKC activation is known to induce the opening of these channels [82]. We can thus presume that K\textsubscript{ATP} channel opening induced by HS could depend on a PKC signalling pathway, potentially through MAPK activation [83,84], although NO [85] and COX-2 [86] are also able to activate these channels.

Thus, further investigations are required to confirm the identity of K\textsubscript{ATP} channels involved in HS preconditioning (i.e., sarcolemmal versus mitochondrial) and also to determine how they are opened and how their opening confers cytoprotection.

4.3.6. Antioxidant enzymes

Antioxidant enzymes could represent potential final end-effectors in the mediation of cardioprotection induced by HS preconditioning. In agreement with Currie et al. [3], we have observed in the rat that HS increases endogenous myocardial catalase activity 24 h later [14]. This antioxidant enzyme appears to be involved in mediating HS-induced cardioprotection, since administration of a catalase inhibitor (3-aminotriazole) prior to ischaemia-reperfusion abolishes the antiarrhythmic effect [14], the improvement in functional recovery [87] and the infarct-sparing effect [88].

Another antioxidant enzyme that can mediate HS preconditioning is manganese superoxide dismutase (Mn-SOD), whose mRNA and protein have been shown to be significantly increased in rat cardiomyocytes 24 h after HS [59,89,90]. Inhibition of Mn-SOD expression by treatment with antisense oligodeoxyribonucleotides completely abolishes the HS-induced tolerance to hypoxia-reoxygenation [89].

4.3.7. Calcium homeostasis

Changes in calcium homeostasis also appear to play a role in the mediation of HS response, being a potential final effector of the cytoprotection. Indeed, HS leads to significantly lower postischaemic mitochondrial calcium content and attenuates submaximal calcium paradox in the isolated rabbit heart [91]. HS-induced myocardial protection is also associated with enhancement of sarcoplasmic reticulum Ca\textsuperscript{2+}-pump activity that maintains net Ca\textsuperscript{2+}-uptake by counterbalancing the enhanced Ca\textsuperscript{2+}-release channel activity produced by ischaemia-reperfusion [92]. Specific studies, measuring intracellular calcium concentration and fluxes, are needed to fully elucidate the exact role of calcium ions in cardioprotection conferred by HS preconditioning.

5. Comparison with other forms of preconditioning

The delayed cardioprotection, induced 24–48 h following HS, appears to be similar to that seen with other forms of preconditioning, which can be broadly classified as non-pharmacological (HS, ischaemia, rapid cardiac pacing and exercise) and pharmacological (endoxotin, cytokines, ROS, NO donors, adenosine receptor agonists, monophosphoryl lipid A and analogs, opioid agonists,….) (for a review see Ref. [33]). Amongst them, ischaemic preconditioning has been extensively studied and appears to induce two distinct
phases of protection: an early phase (lasting for 2–3 h), followed by a delayed one (after 24–96 h) [2].

As for HS, delayed ischaemic preconditioning protects the myocardium against infarction [93], stunning [94], arrhythmias [95] and endothelial dysfunction [96]. The other forms of preconditioning are also able to induce an infarct-sparing effect.

Although the signal transduction pathways underlying all these preconditionings are largely unknown, recent data suggest similarities and common key actors have been identified. There is now convincing evidence that NO and ROS serve as chemical signals triggering development of the preconditioning phenomenon, that PKC is essential for its genesis and that several proteins, such as iNOS, COX-2, antioxidant enzymes, Hsps and K_ATP channels, are possible mediators of the cytoprotection induced. The time course of delayed cardioprotection induced by other forms of preconditioning is also suggestive of a mechanism involving new protein synthesis. However, HS preconditioning only induces delayed cardioprotection [97], suggesting that it is exclusively dependent on new protein synthesis, a characteristic which could ultimately represent an advantage for clinical use.

The shift of the heart to a defensive phenotype is a complex response requiring the coordinated activation of multiple genes. Unravelling the complexity of this polygenetic phenotypic change will likely be a challenge for years to come [33].

6. HS preconditioning and potential therapeutic benefits

The protective HS preconditioning phenomenon is likely to benefit patients suffering repeated ischaemic episodes or at reperfusion following ischaemia. As discussed below, myocardial protection could be obtained either by mimicking HS preconditioning through pharmacological agents or by direct application.

6.1. Pharmacological preconditioning

Therapeutic approaches mimicking HS preconditioning seem feasible today. As previously described, NO and K_ATP potassium channels are some important actors of the preconditioning phenomenon. A sustained cardioprotection similar to that afforded by HS preconditioning can be induced pharmacologically with NO donors and K_ATP channel openers. Thus, nicorandil, which possesses both properties, is an effective anti-anginal agent that can protect the heart in a preconditioning fashion [98]. Various pharmaceutical companies are currently investigating K_ATP channel openers designed to mimic preconditioning. Delayed preconditioning effects of volatile anaesthetics and opioids are also under clinical investigation [99]. Another pharmacological preconditioning agent is adenosine, which is currently used in cardioplegic solutions during cardiopulmonary bypass [100]. However, the clinical use of this agent is only related to its acute cardioprotective effects, and a possible application for its delayed cardioprotective properties remains to be described.

Exploiting the cytoprotective properties of Hsps could also represent a future therapeutic approach. In rodent myocardium, Hsp70 gene transfection is achieved through intracoronary or intravenous injection or by direct injection of naked plasmid or virus liposome. The development of these techniques for clinical use has therapeutic potential [101]. A recently introduced cytoprotective hydroxylamine derivative, bimoclomol, facilitates the formation of all major Hsps, in particular Hsp70, in eukaryotic cells by inducing or amplifying expression of their genes [102]. This compound has been shown to increase cardiomyocyte survival [103] and to protect the rat heart against ischaemia-reperfusion when orally administered 6 h earlier [104]. Interestingly, the beneficial effects of bimoclomol appear only under stress conditions and depend at least in part on its Hsp-coinducer property. This nontoxic drug, which is under Phase II clinical trials, has tremendous therapeutic potential [102,105].

6.2. Performing HS

HS preconditioning can also be directly applied in order to induce protection in specific situations such as transplantation and grafting. In the rat, prior HS has been shown to protect the heart by increasing functional recovery and decreasing cellular necrosis after a cold ischaemia in a protocol mimicking that of heart preservation for transplantation [106]. Similarly, when rat skeletal myoblasts and cardiomyocytes are grafted into the heart for cardiac repair, graft cell survival is enhanced by prior HS [107,108]. Thus, HS could be useful in graft cell survival and in heart preservation protocols for transplantation.

In conclusion, a better understanding of endogenous cardioprotective mechanisms based on experimental investigation could lead to carefully conducted clinical studies comparing the relative effectiveness of this protection with more conventional therapeutic strategies. The identification of the cellular basis of the HS phenomenon should provide a conceptual framework for developing novel therapeutic strategies aimed at mimicking its cardioprotective effects with pharmacological agents or genetic approaches that can maintain the heart in a sustained or chronic defensive state. Although only few potential pharmacological approaches to protection seem feasible at present, we can hope that they will be rapidly developed in the upcoming years, leading to additional myocardial salvage of the reperfused myocardium.

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